# 3A Assembly (Digestion and Ligation)

## Aim

To assemble two BioBricks into a new vector.

## Procedure

### Digestion

1. Prepare following Master Mixes

Table 1: Master Mixes

| **Enzyme Master Mix for Plasmid Backbone (25$\mu$l total, for 5 runs)** | **Enzyme Master Mix for Part A (BioBrick on the 5′ end)** | **Enzyme Master Mix for Part B (BioBrick on the 3′ end)** |
|---|---|---|
| 5 $\mu$l NEB Buffer 2 | 5 $\mu$l NEB Buffer 2 | 5 $\mu$l NEB Buffer 2 |
| 0.5 $\mu$l BSA | 0.5 $\mu$l BSA | 0.5 $\mu$l BSA |
| 0.5 $\mu$l EcoRI-HF | 0.5 $\mu$l EcoRI-HF | 0.5 $\mu$l XbaI |
| 0.5 $\mu$l PstI | 0.5 $\mu$l SpeI | 0.5 $\mu$l PstI |
| 18 $\mu$l $dH_2O$ | 18.5 $\mu$l $dH_2O$ | 18.5 $\mu$l $dH_2O$ |

2. Digest Plasmid Backbone
   - Add 4 $\mu$l linearized plasmid backbone (25 ng/$\mu$l for 100 ng total)
   - Add 4 $\mu$l of Enzyme Master Mix
3. Digest Part A
   - Add 4 $\mu$l Part A (25 ng/$\mu$l for 100 ng total)
   - Add 4 $\mu$l of Enzyme Master Mix
4. Digest Part B
   - Add 4 $\mu$l Part B (25 ng/$\mu$l for 100 ng total)
   - Add 4 $\mu$l of Enzyme Master Mix
5. Digest all three reactions at 37 °C for 60 min, heat kill 80 °C at 20 min

### Ligation

1. Add 2$\mu$l of digested Plasmid Backbone (25 ng)
2. Add equimolar amount of Part A (EcoRI-HF SpeI digested) fragment ($< 3$ $\mu$l)*
3. Add equimolar amount of Part B (XbaI PstI digested fragment) ($< 3$ $\mu$l)*
4. Add 1 $\mu$l T4 DNA ligase buffer.
5. Add 0.5 $\mu$l T4 DNA ligase
6. Add water to 10 $\mu$l

7. Ligate at RT for 10 min, heat kill 80 °C/20 min
8. Transform with 1-2 $\mu$l of product

* Easiest to calculate using `http://www.insilico.uni-duesseldorf.de/Lig_Input.html`
Use a 1:3 vector:insert ratio

## Sources

This protocol is modified from the 3A Assembly protocol at igem.org:
`http://parts.igem.org/Help:Assembly/3A_Assembly`
N.B. We used exceedingly more DNA than iGEM did - DNA volumes of 500 - 1000 $\mu$l.